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(71) Applicant (*for all designated States except US*): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH [US/US]; Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): LIPMAN, David, J. [US/US]; 1818 Greenplace Terrace, Rockville, MD 20850 (US).

(74) Agent: WETHERELL, John, R., Jr.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).

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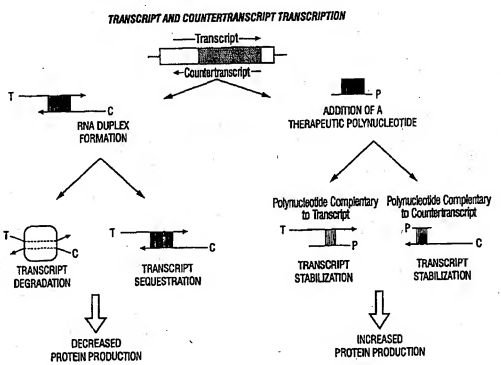

(57) Abstract

A method is described wherein polynucleotides which inhibit RNA destabilization and nuclear sequestration enhance cytoplasmic translocation of stabilized RNA and increase protein expression.

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POLYNUCLEOTIDE INHIBITION OF RNA DESTABILIZATION AND SEQUESTRATION

FIELD OF THE INVENTION

The present invention relates to a method of regulating RNA stability. The invention more specifically relates to the use of polynucleotides to increase the stability of RNA transcripts in order to augment expression of the target gene product.

BACKGROUND OF THE INVENTION

A variety of mechanisms are available in eukaryotic cells for regulating gene expression such that each gene product is produced at appropriate times and in appropriate quantities. It is well established that a significant amount of control over gene expression can be exerted at the level of RNA processing and RNA stability. Recent evidence suggests a role for antisense RNA transcripts in RNA destabilization and nuclear sequestration, a consequence of which is down-regulation of protein expression.

Countertranscript (*i.e.*, antisense) RNA is encoded by the complementary strand of a gene. Countertranscripts are sometimes found in different tissues or developmental stages than their corresponding transcript (*i.e.*, sense) RNA and thus a regulatory role for endogenous countertranscripts has been proposed. Examples of regulation of gene expression by endogenous countertranscripts have been described for nematode, dictyostelium, and prokaryotes. Moreover, studies on the regulation of the countertranscripts of Wilms tumor suppressor, *eif2*- a, and *myc* show that when the sense transcript is up-regulated, the levels of countertranscript decrease, and when the sense transcript is down-regulated, the countertranscript accumulates.

Further evidence suggests that conserved regions in 3' untranslated regions (UTRs) are required for regulation of transcript RNA stability. Typically, deletion of these regions render the transcript RNA unresponsive to regulatory signals which normally lead to destabilization. Ho *et al.*, *J. Biol. Chem.* 270:10084-10090 (1995). Conversely, introduction of these regions into reporter RNAs make them responsive to regulated destabilization. McGowan *et al.*, *J. Biol. Chem.* 272:1331-1337 (1997). Conserved regions in 5' UTRs and coding regions have also been implicated in regulation of mRNA stability. Roy *et al.*, *Nucleic Acids Res.* 20:5753-5762 (1992); Wellington *et al.*, *Mol. Cell. Biol.* 13:5034-5042 (1993).

Double-stranded RNA adenine deaminase (DSRAD) has been implicated in the recognition and destabilization of transcript RNA base-paired with its countertranscript. Kimelman *et al.*, *Cell* 59:687-696 (1989). The modification efficiency of DSRAD has also been shown to decrease exponentially as the length of RNA duplex drops below 100 base pairs. Thus, the destabilization pathway appears to be sensitive to perturbations in the sequence of conserved regions necessary for transcript-countertranscript interactions.

In addition to promoting RNA destabilization, transcript-countertranscript interactions also appear to promote nuclear sequestration of RNA. All known cellular RNA species exit the nucleus through nuclear pore complexes (NPCs). Nakielny *et al.*, *RNA Transport* in Ann. Rev. Neurosci., 20:269-301 (1997). RNA transport through the NPC is mediated by proteins associated with the RNA molecule. These proteins appear to utilize signal-dependent, receptor mediated pathways to transport RNA molecules from the nucleus to the cytoplasm. In addition, RNA which has been processed by 5' capping, 3' polyadenylation and intron removal, is transported from the nucleus more efficiently then unprocessed RNA. Therefore, transcript-countertranscript duplexes promote nuclear accumulation of RNA by interfering with signal-dependent receptor mediated NPC transport and by inhibiting mechanisms required for RNA post-transcriptional processing. Consequently, transcript-countertranscript interactions result in down-regulation of expression of the encoded protein product.

SUMMARY OF THE INVENTION

The present invention is based on the fundamental counterintuitive discovery that defined polynucleotides can prevent transcript-countertranscript interactions and inhibit the degradation and nuclear sequestration of transcript RNA. Thus, the present invention provides methods which can enhance expression of a target protein encoded by transcript RNA.

Under normal conditions long RNA duplexes formed by transcript-countertranscript interactions promote RNA destabilization and nuclear sequestration. The invention provides a method for the inhibition of transcript RNA destabilization and nuclear sequestration through the use of polynucleotides. The polynucleotides are designed such that they interfere with countertranscript annealing to transcript RNA and, thereby, prevent degradation and nuclear accumulation of the targeted transcript. Consequently, the method of the present invention is unexpectedly useful in enhancing translation and augmenting protein expression.

It is an object of the present invention to provide agents in the form of polynucleotides which are effective for enhancing protein expression in target cells and tissues. In accomplishing these and other objects, there has been provided, in accordance with one aspect of the present invention, a method of using polynucleotides to enhance protein expression in targeted tissues where such enhancement is necessary and applicable.

In another aspect of the invention, an expression vector containing in operable linkage a polynucleotide which inhibits destabilization and nuclear sequestration of targeted transcript RNA is provided. The expression vector can be used to stably express polynucleotides in cells or tissue for the purpose of enhancing protein expression.

In yet another aspect of the invention, a method of enhancing translation of mRNA in a patient tissue comprising administering to the patient tissue a therapeutically effective amount of a polynucleotide which inhibits destabilization of transcript RNA is provided. The method includes administering the polynucleotide *in vivo* or *ex vivo*.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 shows a diagram of the mechanism by which the addition of therapeutic polynucleotides can inhibit the destabilization of RNA transcripts and enhance protein expression. Transcript (T) -countertranscript (C) duplex formation promotes the degradation and nuclear sequestration of transcript RNA. The addition of a polynucleotide complementary to a transcript or countertranscript inhibits transcript RNA degradation and sequestration and promotes increased protein production.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention originated from studies on RNA stability and the effect of RNA stability on protein expression. The inventors describe a method for using short polynucleotides to enhance protein expression by preventing transcript-countertranscript interactions which result in long duplex formation.

During their investigation the inventor discovered that a substantial fraction of vertebrate RNAs contain long conserved sequences in their untranslated regions as well as highly conserved sequences without silent changes in their protein coding regions. These conserved regions are largely comprised of unique sequence within the genome and

appear to be associated with regulation of RNA stability. Further, the inventor has identified these highly conserved sequences in the coding regions and 5' and 3' UTR's of orthologous vertebrate RNAs. A representative sample from a comparison of human and mouse orthologs is shown in Table I. A mechanism is proposed wherein conserved sequences contained in transcript RNA form long, highly complementary duplexes with the corresponding region of the corresponding countertranscript RNA. Further, it is proposed that such duplexes are targeted by post-transcriptional regulatory mechanisms which promote long duplex degradation or nuclear sequestration of the transcript-countertranscript duplex, thereby preventing transcript translation and inhibiting protein production. This mechanism lends itself to modulation by compositions which disrupt or interfere with long duplex formation.

Polynucleotide Stabilization of Transcript RNA

In a preferred embodiment, the present invention relates to a method for enhancing transcript RNA stability in a cell by contacting the cell with a polynucleotide which inhibits transcript RNA degradation. The polynucleotides prevent long transcript-countertranscript duplex formation which results in enhanced transcript RNA stability and cytoplasmic translocation.

"Transcript RNA", as used herein, is RNA which contains nucleotide sequence encoding a protein product. Preferably, the transcript RNA is messenger RNA (mRNA). "mRNA", as used herein, is a single-stranded RNA molecule that specifies the amino acid sequence of one or more polypeptide chains. In addition, transcript RNA can be heterogenous nuclear RNA (hnRNA) or masked RNA. "hnRNA", as the term is used herein, represents the primary transcripts of RNA polymerase II and includes precursors of all messenger RNAs from which introns are removed by splicing. hnRNA are extensively processed to give mRNA which is exported to the cytoplasm where protein synthesis occurs. This processing may include the addition of a 5'-linked 7-methyl-guanylate "cap" at the 5' end and a sequence of adenylate groups at the 3' end, the poly A "tail", as well as the removal of any introns and the splicing together of exons. "Masked RNA", as

used herein, is any form of mRNA which is present in inactive form. More specifically, masked RNA constitutes a store of maternal information for protein synthesis that is unmasked (derepressed) during the early stages of morphogenesis.

"Countertranscript", as used herein, is that sequence which is transcribed from the non-coding strand of a gene sequence. Countertranscript RNA can be fully complementary to the transcript RNA. However, it is understood that any countertranscript capable of generating duplex structures with transcript RNA such that the duplex structures result in transcript RNA degradation or sequestration are included in the method of the present invention.

The term "transcript RNA degradation", as used herein, is any mechanism which initiates RNA destabilization through the recognition of duplex RNA sequences resulting in the loss of transcript RNA integrity. Preferably, the mechanism can involve double-strand RNA adenine deaminase (DSRAD), however, any activity or mechanism which acts to degrade or destabilize the transcript RNA is included in the present method.

"Transcript RNA sequestration", as used herein, is any mechanism which relies on RNA duplex formation to inhibit or prevent transcript RNA translocation from the nucleus to the cytoplasm. These mechanisms include, but are not limited to, those that prevent interaction of the transcript RNA with export factors required for nuclear pore complex (NPC) mediated transport. Further, duplex formation can prevent normal post-transcriptional RNA processing resulting in the nuclear accumulation of unprocessed transcript. Other mechanisms which promote RNA nuclear sequestration will be known to those of skill in the art.

Nucleic acid sequences of the invention can be obtained by several methods. For example, the sequence can be isolated using hybridization or computer-based techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences;

2) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features; 3) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest; 4) computer searches of sequence databases for similar sequences; and 5) differential screening of a subtracted DNA library. Based upon the size of the human genome, statistical studies show that a DNA segment approximately 14-15 base pairs long will have a unique sequence in the genome.

According to the method of the present invention, the sequence of a suitable polynucleotide can be obtained, for example, by comparing the sequences of orthologous genes, or the transcripts or countertranscripts of orthologous genes, and identifying highly conserved regions within the orthologous sequences. Preferably, highly conserved regions contained within orthologs, or transcripts and countertranscripts of orthologs, can be used to design polynucleotides useful for inhibiting transcript-countertranscript interactions. As used herein, an "orthologous sequence" is that in which sequence homology is retained or conserved between species. Two gene sequences from different organisms are orthologs if they derived from the same gene in the closest ancestor to the two organisms. For example, all vertebrate globin genes are homologous in that their genes are derived from a single globin gene in early vertebrates. Consequently, human and horse a-globin genes, and transcripts or countertranscripts encoded therefrom, are orthologous because they have a common ancestor and share significant sequence homology. Therefore, polynucleotides useful for the method of the present invention can be designed such that they contain nucleic acid sequence which is, for example, wholly or partially complementary to conserved sequences identified from orthologous sequences. Preferably, the polynucleotide is designed such that it is complementary to a coding region or the 5' or 3' UTR of a transcript or countertranscript identified from the aforementioned orthologous sequences. However, it is understood that any portion of a gene or transcript or countertranscript can encode sequences which are useful for designing polynucleotides according to the method of the present invention.

Similarity in nucleic acid sequences may be determined by procedures and algorithms which are well-known in the art. Such procedures and algorithms include, for example, a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignmnet by Genetic ALgorithm) and WHAT-IF.

In one aspect of the invention, the polynucleotide is designed to be complementary to the transcript RNA of a gene sequence. In another aspect of the invention, the polynucleotide is designed to be complementary to the countertranscript RNA of a gene sequence.

Polynucleotides useful in the context of the present invention include both short sequences of RNA or DNA, usually 10-50 bases in length as well as longer sequences of RNA or DNA that may exceed the length of the target transcript sequence itself. Thus, it is understood that any polynucleotide which inhibits, abrogates or interferes with endogenous mechanisms for transcript RNA destabilization or sequestration is hereby incorporated in the present method. Preferably, polynucleotides useful for the present invention are complementary to specific regions of a corresponding target RNA. Such target RNA can be either the transcript or countertranscript RNA. Hybridization of

polynucleotides to their target transcripts can be highly specific as a result of complementary base pairing. The capability of polynucleotides to hybridize to a target transcript is affected by such parameters as length, chemical modification and secondary structure of the transcript which can influence polynucleotide access to the target site. See Stein *et al*, *Cancer Research* 48:2659 (1988). A stabilizing RNA polynucleotide can be produced in a cell by introducing a DNA segment that codes for the polynucleotide into the cell. A stabilizing polynucleotide can also be introduced to a cell by adding the polynucleotide to the environment of the cell such that the cell takes up the polynucleotide directly. The latter route is preferred for the shorter polynucleotides of up to about 20 bases in length.

In another aspect of the invention, it is envisioned that long polynucleotides which may exceed the length of the target transcript can be used to prevent transcript RNA destabilization. Further, such long polynucleotides can include multiple regions complementary to multiple corresponding regions in a target transcript. For example, one or more highly conserved regions in a transcript or countertranscript can be targeted by a polynucleotide containing regions which are complementary to at least one an preferably more of the highly conserved regions. Thus, a single long polynucleotide can prevent transcript RNA destabilization by containing at least one and preferably multiple regions of complementarity of the targeted transcript.

In yet another aspect of the invention, a long polynucleotide can contain multiple regions of complementarity to a highly conserved sequence in one or more transcript or countertranscript RNAs. Such a polynucleotide can contain, for example, one or more regions complementary to highly conserved regions in a target transcript RNA and, in addition, one or more regions complementary to highly conserved regions in a second target transcript RNA. According to the method of the present invention, a long polynucleotide is useful for the inhibition of transcript destabilization of one or more transcript RNAs. For example, tumorigenic cells can be treated with a vector expressing a polynucleotide with multiple regions of homology to transcripts encoding polypeptides

involved in regulating apoptosis. Such polynucleotides can inhibit transcript destabilization of one or more different transcripts simultaneously or individually. Thus, a polynucleotide can contain regions of complementarity to highly conserved regions in, for example, a transcript encoding the p53 protein and highly conserved regions in, for example, a transcript encoding the c-myc protein. The presence of a single polynucleotide with such regions can inhibit the destabilization of multiple apoptosis-promoting transcripts thereby increasing the cellular concentration of the encoded protein and resulting in programmed cell death in tumorigenic cells so treated.

In selecting the preferred length for a given polynucleotide, various factors should be considered to achieve the most favorable characteristics. In one aspect, polynucleotides of the present invention are at least 15 bp in length and preferably about 15 to about 100 bp in length. More preferably, the polynucleotides are about 15 bp to about 80 bp in length and even more preferably, the polynucleotides of the present invention are about 15 to about 60 bp in length. Shorter polynucleotides such as 10-to under 15-mers, while offering higher cell penetration, have lower gene specificity. In contrast, while longer polynucleotides of 20-30 bases offer better specificity, they show decreased uptake kinetics into cells. See Stein *et al.*, "Oligodeoxynucleotides - Antisense Inhibitors of Gene Expression" Cohen, ed. McMillan Press, London (1988). Accessibility to transcript or countertranscript RNA target sequences also is of importance and, therefore, loop-forming regions and orthologous sequences in targeted RNAs offer promising targets. In this disclosure the term "polynucleotide" encompasses both oligomeric nucleic acid moieties of the type found in nature, such as the deoxyribonucleotide and ribonucleotide structures of DNA and RNA, and man-made analogues which are capable of binding to nucleic acids found in nature. The polynucleotides of the present invention can be based upon ribonucleotide or deoxyribonucleotide monomers linked by phosphodiester bonds, or by analogues linked by methyl phosphonate, phosphorothioate, or other bonds. They may also comprise monomer moieties which have altered base structures or other modifications, but which still retain the ability to bind to naturally occurring transcript and countertranscript RNA structures. Such polynucleotides may

be prepared by methods well-known in the art, for instance using commercially available machines and reagents such as those available from Perkin-Elmer/Applied Biosystems (Foster City, CA). For example, polynucleotides specific to a targeted transcript are synthesized according to standard methodology. Phosphorothioate modified DNA polynucleotides typically are synthesized on automated DNA synthesizers available from a variety of manufacturers. These instruments are capable of synthesizing nanomole amounts of polynucleotides as long as 100 nucleotides. Shorter polynucleotides synthesized by modern instruments are often suitable for use without further purification. If necessary, polynucleotides may be purified by polyacrylamide gel electrophoresis or reverse phase chromatography. See Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Vol. 2, Chapter 11, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

Phosphodiester-linked polynucleotides are particularly susceptible to the action of nucleases in serum or inside cells, and therefore in a preferred embodiment, the polynucleotides of the present invention are phosphorothioate or methyl phosphonate-linked analogues, which have been shown to be nuclease-resistant. Persons of ordinary skill in this art can easily select other linkages for use in the invention. These modifications also may be designed to improve the cellular uptake and stability of the polynucleotides.

An appropriate carrier for administration of a polynucleotide can include, for example, vectors, antibodies, pharmacologic compositions, binding or homing proteins, or viral delivery systems to enrich for the sequence into the target cell or tissue. A polynucleotide of the present invention can be coupled to, for example, a binding protein which recognizes endothelial cells or tumor cells. Following administration, a polynucleotide of the present invention can be targeted to a recipient cell or tissue such that enhanced expression of, for example, cytokines, transcription factors, G-protein coupled receptors, tumor suppressor proteins and apoptosis initiation proteins can occur.

Polynucleotide Expression

In another embodiment of the invention, an expression vector containing in operable linkage a polynucleotide which inhibits destabilization or nuclear sequestration of transcript RNA is disclosed. Preferably, the polynucleotide is an RNA polynucleotide produced by introducing an expression construct into a target cell. The RNA molecule thus produced is chosen to have the capability to hybridize to a target transcript RNA or its countertranscript. The polynucleotides which have the capability to hybridize with RNA targets can enhance expression of corresponding gene products by multiple mechanisms. Such molecules that have this capability can inhibit degradation of the target mRNA and thereby enhance translation.

As used herein, the term "operable linkage" refers to functional linkage between a promoter sequence and the structural gene regulated by the promoter nucleic acid sequence. The operably linked promoter controls the expression of the polypeptide encoded by the structural gene, or in a preferred embodiment of the invention, the heterologous gene nucleic acid sequences. The orientation or placement of the elements of the vector is not strict, so long as the "operable linkage" requirement is fulfilled for control of and expression of the nucleic acid sequences.

A polynucleotide in the form of complementary RNA may be introduced to a cell by its expression within the cell from a standard DNA expression vector. Target DNA sequences can be cloned from standard plasmids into expression vectors, which expression vectors have characteristics permitting higher levels of, or more efficient expression of, the resident polynucleotides. At a minimum, these constructs require a prokaryotic or eukaryotic promoter sequence which initiates transcription of the inserted DNA sequences. A preferred expression vector is one where the expression is inducible to high levels. High levels of expression can be accomplished by the addition of a regulatory region which provides increased transcription of downstream sequences in the appropriate host cell. See Sambrook *et al.*, Vol. 3, Chapter 16 (1989). For example, RNA polynucleotide expression vectors can be constructed using the polymerase chain

reaction (PCR) to amplify appropriate fragments from single-stranded cDNA of a plasmid in which target DNA has been incorporated. Polynucleotide synthesis and purification techniques are described in Sambrook *et al.* and Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology* (Wiley Interscience 1987) (hereafter "Ausubel"), respectively. The PCR procedure is performed via well-known methodology. See, for example, Ausubel, and Bangham, "The Polymerase Chain Reaction: Getting Started," *in Protocols in Molecular Genetics* (Humana Press, 1991). Moreover, PCR kits can be purchased from companies such as Stratagene Cloning Systems (La Jolla, CA) and Invitrogen (San Diego, CA).

The products of PCR are subcloned into cloning vectors. In this context, a "cloning vector" is a DNA molecule, such as a plasmid, cosmid or bacteriophage, that can replicate autonomously in a host prokaryotic or eukaryotic cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion without loss of an essential biological function of the vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Suitable cloning vectors are described by Sambrook *et al.*, Ausubel, and Brown (ed.), *Molecular Biology LabFax* (Academic Press 1991). Cloning vectors can be obtained, for example, from GIBCO/BRL (Gaithersburg, MD), Clontech Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI), Stratagene Cloning Systems (La Jolla, CA), Invitrogen (San Diego, CA), and the American Type Culture Collection (Rockville, MD).

Cloned polynucleotides are amplified by transforming competent bacterial cells with a cloning vector and growing the bacterial host cells in the presence of the appropriate antibiotic. See, for example, Sambrook *et al.*, and Ausubel. PCR is then used to screen bacterial host cells for the appropriate clones. The use of PCR for bacterial host cells is described, for example, by Hofmann *et al.*, "Sequencing DNA Amplified Directly from a Bacterial Colony," in *PCR Protocols and Applications*, White (ed.), pages 205-210

(Humana Press 1993), and by Cooper *et al.*, "PCR-Based Full-Length cDNA Cloning Utilizing the Universal-Adaptor/Specific DOS Primer-Pair Strategy," *Id.* at pages 305-316.

Art-known methods for expressing an exogenous polynucleotide in a cell of a mammal can be used to express RNA polynucleotides complementary to a target RNA transcript or a target RNA countertranscript such that the polynucleotide prevents duplex formation. Typically, a nucleic acid encoding a polynucleotide of the present invention is operably linked to a mammalian-active promoter. A "mammalian active" promoter is a nucleic acid sequence that directs transcription in a mammalian cell (e.g., a promoter of a mammal or a virus that infects a mammal). If desired, the promoter can be cell-specific, tissue-specific, or stage-specific in order to express the polynucleotide with increased specificity. Numerous genetic constructs and methods for expressing heterologous genes in cells of mammals are known in the art and are suitable for use in the invention. For example, polynucleotide expression can be accomplished with conventional gene therapy methods, such as those that employ viral vectors (e.g., vectors derived from retroviruses, adenoviruses, herpes viruses, vaccinia viruses, polio viruses, sindbis viruses, or adeno-associated viruses).

For a mammalian host, the transcriptional and translational regulatory signals preferably are derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al.*, *J. Molec. Appl. Genet.* 1: 273 (1982)); the TK promoter of Herpes virus (McKnight, *Cell* 31: 355 (1982)); the SV40 early promoter

(Benoist *et al.*, *Nature* 290: 304 (1981); the Rous sarcoma virus promoter (Gorman *et al.*, *Proc. Nat'l Acad. Sci. USA* 79: 6777 (1982)); and the cytomegalovirus promoter (Foecking *et al.*, *Gene* 45: 101 (1980)).

A vector for introducing at least one polynucleotide, complementary to a transcript or its countertranscript, into a cell by expression from a DNA is the vector pRc/CMV (Invitrogen, San Diego, CA), which provides a high level of constitutive transcription from mammalian enhancer-promoter sequences.

Another possible method by which polynucleotide sequences may be exploited is via gene therapy. Virus-like vectors, usually derived from retroviruses, may prove useful as vehicles for the importation and expression of polynucleotide constructs in human cells. Generally, such vectors are non-replicative *in vivo*, precluding any unintended infection of non-target cells. In such cases, helper cell lines are provided which supply the missing replicative functions *in vitro*, thereby permitting amplification and packaging of the vector encoding the polynucleotide. A further precaution against accidental infection of non-target cells involves the use of target cell-specific regulatory sequences. When under the control of such sequences, polynucleotide constructs would not be expressed in normal tissues.

Polynucleotides for Therapeutic Use

In another embodiment, a method for increasing cytoplasmic transcript RNA in a subject comprising administering a therapeutically effective amount of a polynucleotide which inhibits degradation or nuclear sequestration of transcript RNA. In one aspect, a pharmaceutical composition containing the polynucleotide of the present invention is administered. Administering the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan and those discussed throughout the specification. The term "subject" means any mammal, preferably a human. The method is useful for the treatment of any condition wherein inhibition of transcript RNA degradation and sequestration would be efficacious. Preferably, the

treatment would be useful in treating disorders in which increased production of a target protein would ameliorate the symptoms of the disorder. For example, tumorigenic cells can be treated with a polynucleotide such that the polynucleotide enhances the production of a protein involved in regulating cellular apoptosis and inhibiting cell growth. Examples of such proteins would include, but not be limited to, ICE (interleukin Ib converting enzyme), BAD, Bax, Bak, NBK, p53, Rb, bcl-x_s, c-myc, Fas, TNF, Hid and WAF1.

The terms "treating", "treatment", and the like are used herein to mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disorder or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse effect attributable to the disorder. "Treating" as used herein covers any treatment of a disorder in a mammal, and includes:

(a) preventing a disorder from occurring in a subject that may be predisposed to a disorder, but has not yet been diagnosed as having it;

(b) inhibiting a disorder, *i.e.*, arresting its development; or

(c) relieving or ameliorating the disorder, e.g., cause regression of the disorder.

Accordingly, "treatment" is intended to mean providing a therapeutically detectable and beneficial effect on a patient afflicted with a disorder the symptoms of which can be ameliorated by administration of a polynucleotide which prevents transcript-countertranscript duplex formation.

The term "effective amount" or "therapeutically effective amount", as used herein, is the amount sufficient to obtain the desired physiological effect, e.g., treatment of a disorder. An effective amount of the vector expressing, for example, a polynucleotide of the invention is generally determined by the physician in each case on the basis of factors normally considered by one skilled in the art to determine appropriate dosages, including

the age, sex, and weight of the subject to be treated, the condition being treated, and the severity of the medical condition being treated.

Administration of a polynucleotide to a subject, either as a naked, synthetic polynucleotide or as part of an expression vector, can be effected via any common route (oral, nasal, buccal, rectal, vaginal, or topical), or by subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Pharmaceutical compositions of the present invention, however, are advantageously administered in the form of injectable compositions. A typical composition for such purpose comprises a pharmaceutically acceptable solvent or diluent and other suitable, physiologic compounds. For instance, the composition may contain polynucleotides and about 10 mg of human serum albumin per milliliter of a phosphate buffer containing NaCl. As much as 700 milligrams of a polynucleotide has been administered intravenously to a patient over a course of 10 days (*i.e.*, 0.05 mg/kg/hour) without signs of toxicity. Sterling, "Systemic Antisense Treatment Reported," *Genetic Engineering News* 12:1, 28 (1992).

Other pharmaceutically acceptable excipients include non-aqueous or aqueous solutions and non-toxic compositions including salts, preservatives, buffers and the like. Examples of non-aqueous solutions are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous solutions include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to routine skills in the art. A preferred pharmaceutical composition for topical administration is a dermal cream or transdermal patch.

Polynucleotides or their expression vectors may be administered by injection as an oily suspension. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides. Moreover, polynucleotides or vectors may be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension also contains stabilizers.

Liposome encapsulation provides an alternative formulation for the administration of polynucleotides and expression vectors. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments. See, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1): S61 (1993), and Kim, *Drugs* 46: 618 (1993). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s). See, for example, Machy *et al., Liposomes in Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm.* 46: 1576 (1989). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes. Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents. Scherphof *et al., Ann. N.Y. Acad. Sci.* 446:368 (1985).

After intravenous administration, conventional liposomes are preferentially phagocytosed into the reticuloendothelial system. However, the reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means. Claassen *et al., Biochim. Biophys. Acta* 802:428 (1984). In addition, incorporation of glycolipid- or polyethelene glycol-derivatised phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system. Allen *et al., Biochim. Biophys. Acta* 1068:133 (1991); Allen *et al., Biochim. Biophys. Acta* 1150:9 (1993) These Stealth® liposomes have an increased circulation time and an improved targeting to tumors in animals. Woodle *et al., Proc. Amer. Assoc. Cancer Res.* 33:2672 (1992). Human clinical trials are in progress, including Phase III clinical trials against Kaposi's sarcoma. Gregoriadis *et al.*, 1993, *Drugs* 45:15).

Polynucleotides and expression vectors can be encapsulated within liposomes using standard techniques. A variety of different liposome compositions and methods for synthesis are known to those of skill in the art. See, for example, U.S. Patent No. 4,844;904, U.S. Patent No. 5,000,959, U.S. Patent No. 4,863,740, and U.S. Patent No. 4,975,282, all of which are hereby incorporated by reference.

Liposomes can be prepared for targeting to particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For instance, antibodies specific to tumor associated antigens may be incorporated into liposomes, together with polynucleotides or expression vectors, to target the liposome more effectively to the tumor cells. See, for example, Zelphati *et al., Antisense Research and Development* 3: 323-338 (1993), describing the use "immunoliposomes" containing polynucleotides for human therapy.

In general, the dosage of administered liposome-encapsulated polynucleotides and vectors will vary depending upon such factors as the patient's age, weight, height, sex,

general medical condition and previous medical history. Dose ranges for particular formulations can be determined by using a suitable animal model.

Polynucleotides, or vectors containing the polynucleotide of the claimed invention, can be administered *ex vivo* by transfer of genetic material to cells, for example, hematopoietic stem cells located outside a subject. *Ex vivo* transfer, as used herein, utilizes available techniques known to those skilled in the art to remove cells from a subject and introduce into these cells a therapeutic amount of genetic material. Following transfer of the genetic material, the transduced cells can be implanted back in the original host subject or into a different host, such as a member of the same or a different species from the donor. For example, hematopoietic stem cells can be removed from a subject, treated with the appropriate polynucleotide and re-introduced into the host subject.

In vivo transfer, as used herein, utilizes available techniques known to those skilled in the art to introduce into a subject a therapeutic amount of genetic material. For example, tumor cells within an organism can be targeted for therapy by polynucleotides of the claimed invention. Polynucleotides which promote expression of a protein involved in regulating apoptosis, for example, can be used to promote programmed cell death in tumorigenic cells.

DNA sequences encoding a polynucleotide specific for a transcript or countertranscript can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the target genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, 1987, *Gene* 56:125), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, 1988, *J. Biol. Chem.*, 263:3521) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein I, or polyhedrin promoters).

A variety of host-expression vector systems may be utilized to express a portion of the target transcript coding sequence. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the polynucleotide sequence; yeast transformed with recombinant yeast expression vectors containing the polynucleotide sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the polynucleotide coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the polynucleotide coding sequence; or animal cell systems infected with recombinant virus expression vectors (*e.g.*, retroviruses, adenovirus, vaccinia virus) containing the polynucleotide coding sequence, or transformed animal cell systems engineered for stable expression.

Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression may be engineered. For example, when using adenovirus expression vectors, the coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. Alternatively, the vaccinia virus 7.5K promoter may be used. (*e.g.*, see, Mackett *et al.*, 1982, *Proc. Natl. Acad. Sci. USA* 79:7415-7419; Mackett *et al.*, 1984, *J. Virol.* 49:857-864; Panicali *et al.*, 1982, *Proc. Natl. Acad. Sci. USA* 79:4927-4931). Of particular interest are vectors based on bovine papilloma virus which have the ability to replicate as extrachromosomal elements (Sarver, *et al.*, 1981, *Mol. Cell. Biol.* 1:486). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as, for example, the neo gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the polynucleotide sequence in host cells (Cone & Mulligan, 1984, *Proc. Natl. Acad. Sci. USA* 81:6349-6353). High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and heat shock promoters.

For long-term, high-yield production of recombinant polynucleotides, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the cDNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, etc.), and a selectable marker. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, *Cell* 11:223),

hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48: 2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, *Cell* 22:817) genes can be employed in tk-, hgprt- or aprt- cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, *Natl. Acad. Sci. USA* 77:3567; O'Hare, *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072; neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, *J. Mol. Biol.* 150:1); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, *Gene* 30:147) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, *Proc. Natl. Acad. Sci. USA* 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory ed.).

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the polynucleotide of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

In a preferred embodiment, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

For a mammalian host, several possible vector systems are available for expression of the polynucleotide specific for a targeted transcript. One class of vectors utilize DNA elements which provide autonomously replicating extra-chromosomal plasmids, derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, or SV40 virus. A second class of vectors include vaccinia virus expression vectors. A third class of vectors relies upon the integration of the desired gene sequences into the host chromosome. Cells which have stably integrated the introduced DNA into their chromosomes may be selected by also introducing one or more markers (*e.g.*, an exogenous gene) which allow selection of host cells which contain the expression vector. The marker may provide for prototropy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper or the like. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by co-transformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements include those described by Okayama, H., *Mol. Cell. Biol.*, 3:280 (1983), and others.

Once the vector or DNA sequence containing the construct has been prepared for expression, the DNA construct may be introduced (transformed) into an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques.

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention are in general administered topically, intravenously, orally or parenterally or as implants, but even rectal use is possible in principle. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249:1527-1533 (1990), which is incorporated herein by reference.

The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the

symptoms of the disease and its complications or enhance protein expression in cells or tissue where it is desirable to do so. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman *et al.* (eds.) (1990) Goodman and Gilman's: *The Pharmacological Basis of Therapeutics*, 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed. (1990), Mack Publishing Co., Easton, Pa., each of which is herein incorporated by reference.

Having thus disclosed exemplary embodiments of the present invention, it should be noted by those skilled in the art that the disclosures are exemplary only and that various other alternatives, adaptations and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein.

EXAMPLE 1

Identification of Highly Conserved Regions in Orthologous Sequences

A sequence comparison of human and mouse orthologous mRNA's was performed using an available database of nucleic acid sequences from both organisms. The data shown in Table I includes a representative sample of human mRNA's with long perfect blocks of identity in the coding regions when aligned with orthologous mouse mRNA. These data further indicate highly conserved regions in 5' and 3' untranslated regions (UTR's) of human and mouse orthologs. The conserved regions contained in orthologous sequences can be used to design polynucleotides of the present invention. The polynucleotides are designed such that their sequence is complementary to a highly conserved region in the transcript or countertranscript of an orthologous sequence. Therefore, the data in Table I can be used to design polynucleotides such that the polynucleotides inhibit transcript RNA degradation and nuclear sequestration by preventing transcript-countertranscript duplex formation between highly conserved regions of orthologous sequences. Administering such a polynucleotide to a subject can serve to enhance the expression of a target protein in that subject for the purpose of treating a disorder. Techniques for administering nucleic acids to a subject are known to those of skill in the art. A polynucleotide of the present invention can be administered to a subject suffering from a cell proliferation disorder such that the polynucleotide increases the expression of proteins involved in regulating apoptosis. Cells treated in such a manner can undergo apoptosis and alleviate symptoms of a cell proliferation disorder.

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Table 1

Examples of Conserved Blocks in Human/Mouse Orthologous mRNAs[a]

	Accession number (human)	Conserved Regions		
		5' UTR length (%)	Coding Region identical blocks	3'UTR length (%)
Immediate-early response protein NOT mRNA	X675918		153, 172, 150	
Human polyposis locus (DP2.5 gene) mRNA	M73548		147, 199	
octamer binding transcription factor 1 (OTF1) mRNA	L2043		116, 125	
homeobox protein hox-c4 (hox-3e) (cp19) mRNA	X07495		124, 136	
acute phase response factor mRNA	L29277		178	69 (96%)
RNA binding protein EWS mRNA	X79233		133	156 (97%)
hnRNP-E2 mRNA	X78136		209	167 (91%)
eukaryotic initiation factor 4AII mRNA	D30655		167, 116	345(96%) 190 (96%)
fibrillin mRNA	L13923		151	484 (87%)
glutamate receptor 2 (HBGR2) mRNA	L20814	158 (85%)	258, 157	202 (98%)
p68 protein mRNA	X52104		175	301 (97%)
thyroid hormone receptor alpha (c-erbA-1) mRNA	X55005	173 (91%)	183	80 (96%)

[a] A representative sample of human mRNAs with long, perfect blocks of identity in the coding regions when aligned with the orthologous mouse mRNA. Conserved regions in 5' and 3' UTR are also indicated when present.

		5' UTR length (%)	Coding Region identical blocks	3'UTR length (%)
S-adenosylmethionine decarboxylase mRNA	M21154	122 (95%) 119 (97%)	92, 139, 134	119 (88%)
sodium- and chloride-dependent taurine transporter mRNA	Z18956		160	69 (94%)
transcription activator ZFX mRNA	X59739		159	575 (86%)
homeo box c8 protein mRNA	M16938	208 (98%)	278	184 (85%) 163 (88%)
leukemia virus receptor 1 (GLVR1) mRNA	L20859	152 (94%)	145	145 (94%) 171 (88%)
very low density lipoprotein receptor mRNA	L20470		112	431 (93%)
nervous-system specific octamer-binding transcription factor n-Oct 3 mRNA	Z11933	60 (97%)	147	
glutamate (NMDA) receptor subunit zeta 1 mRNA	D13515		139	78 (96%)
voltage-dependent L-type Ca channel alpha 1 subunit mRNA	Z34822		137	84 (99%) 101 (95%)

CLAIMS

What is claimed is:

1. A method for enhancing transcript RNA stability in a cell, the method comprising contacting the cell with a polynucleotide which inhibits transcript RNA degradation.

2. The method of claim 1, wherein the RNA is selected from the group consisting of mRNA, hnRNA and masked RNA.

3. The method of claim 1, wherein the polynucleotide is complementary to the transcript RNA.

4. The method of claim 3, wherein the polynucleotide is complementary to an untranslated region (UTR) of the transcript RNA.

5. The method of claim 3, wherein the polynucleotide is complementary to a translated region of the transcript RNA.

6. The method of claim 3, wherein the polynucleotide is complementary to a countertranscript to the transcript RNA.

7. The method of claim 6, wherein the polynucleotide is complementary to a region of the countertranscript that is complementary to an UTR of the transcript RNA.

8. The method of claim 6, wherein the polynucleotide is complementary to a region of the countertranscript that is complementary to a translated region of the transcript RNA.

9. The method of claim 6, wherein the polynucleotide is complementary to a conserved region within an orthologous sequence.

10. The method of claim 9, wherein the polynucleotide contains one or more regions complementary to one or more conserved regions within an orthologous sequence.

11. The method of claim 1, wherein the polynucleotide is DNA.

12. The method of claim 11, wherein the polynucleotide is at least 15 nucleotides in length.

13. The method of claim 12, wherein the polynucleotide is from about 15 nucleotides to 100 nucleotides in length.

14. The method of claim 12, wherein the polynucleotide is from about 15 nucleotides to 80 nucleotides in length.

15. The method of claim 12, wherein the polynucleotide is from about 15 nucleotides to 60 nucleotides in length.

16. The method of claim 1, wherein the polynucleotide is RNA.

17. The method of claim 1, wherein the cell is a eukaryotic cell.

18. The method of claim 17, wherein the cell is a mammalian cell.

19. The method of claim 18, wherein the cell is a human cell.

20. An expression vector containing in operable linkage a polynucleotide which inhibits degradation or nuclear sequestration of transcript RNA.

21. A cell containing the vector of claim 20.

22. A method of increasing cytoplasmic transcript RNA in a subject comprising administering a therapeutically effective amount of a polynucleotide which inhibits degradation or nuclear sequestration of transcript RNA.

23. The method of claim 22, wherein the subject has a transcript RNA translation deficiency disorder.

24. The method of claim 22, wherein the polynucleotide is administered *in vivo*.

25. The method of claim 22, wherein the polynucleotide is administered to tumor cells.

26. The method of claim 22, wherein the polynucleotide is administered *ex vivo*.

27. The method of claim 26, wherein the polynucleotide is administered to hematopoietic stem cells.

28. A method of increasing cytoplasmic transcript RNA concentration in a cell, the method comprising contacting the cell with a polynucleotide which inhibits nuclear sequestration of transcript RNA.

29. The method of claim 28, wherein RNA is selected from the group consisting of mRNA, hnRNA and masked RNA.

30. The method of claim 28, wherein the polynucleotide is complementary to the transcript RNA.

31. The method of claim 30, wherein the polynucleotide is complementary to an untranslated region (UTR) of the transcript RNA.

32. The method of claim 30, wherein the polynucleotide is complementary to a translated region of the transcript RNA.

33. The method of claim 30, wherein the polynucleotide is complementary to a countertranscript to the transcript RNA.

34. The method of claim 33, wherein the polynucleotide is complementary to a region of the countertranscript that is complementary to an UTR of the transcript RNA.

35. The method of claim 33, wherein the polynucleotide is complementary to a region of the countertranscript that is complementary to a translated region of the transcript RNA.

36. The method of claim 33, wherein the polynucleotide is complementary to a conserved region within an orthologous sequence.

37. The method of claim 28, wherein the polynucleotide is DNA.

38. The method of claim 37, wherein the polynucleotide is at least 15 nucleotides in length.

39. The method of claim 38, wherein the polynucleotide is from about 15 nucleotides to 100 nucleotides in length.

40. The method of claim 38, wherein the polynucleotide is from about 15 nucleotides to 80 nucleotides in length.

41. The method of claim 38, wherein the polynucleotide is from about 15 nucleotides to 60 nucleotides in length.

42. The method of claim 28, wherein the polynucleotide is RNA.

43. The method of claim 28, wherein the cell is a eukaryotic cell.

44. The method of claim 28, wherein the cell is a mammalian cell.

45. The method of claim 28, wherein the cell is a human cell.

46. The method of claim 1 or claim 28, wherein the cell is a plant cell.

TRANSCRIPT AND COUNTERTRANSCRIPT TRANSCRIPTION
Transcript
Countertranscript
T
C
RNA DUPLEX FORMATION
ADDITION OF A THERAPEUTIC POLYNUCLEOTIDE
P
Polynucleotide Complentary to Transcript
Polynucleotide Complentary to Countertranscript
TRANSCRIPT STABILIZATION
TRANSCRIPT SEQUESTRATION
TRANSCRIPT DEGRADATION
DECREASED PROTEIN PRODUCTION
INCREASED PROTEIN PRODUCTION

FIG. 1

INTERNATIONAL SEARCH REPORT

Int. Ional Application No
PCT/US 98/17261

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/67 C12N15/11 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 11085 A (UNIV MASSACHUSETTS) 27 March 1997 see page 3, line 11 - line 34 see page 13, line 6 - line 24 see page 15, line 32 - line 34	1-4, 11-19, 22,24, 26-31, 37-45
Y	see claims 28-30	1-46
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 1999	18/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016	Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

inte onal Application No
PCT/US 98/17261

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	DURET, L. ET AL.: "Strong conservation of non-coding sequences during vertebrates evolution: potential involvement in post-transcriptional regulation of gene expression" NUCLEIC ACIDS RESEARCH., vol. 21, 1993, pages 2315-2322, XP002089028 see abstract see page 2320, right-hand column, last paragraph - page 2321, right-hand column, paragraph 3 ---	1-46
X	DELAFONTAINE, P. ET AL.: "Regulation of vascular smooth muscle cell insuline-like growth factor I receptors by phosphorothioate oligonucleotides" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 270, 16 June 1995, pages 14383-14388, XP002089029 MD US see page 14386, right-hand column, line 7; figures 6A,B see page 14387, left-hand column, paragraph 2 - right-hand column, paragraph 2 ---	1,2,6,8, 11-15, 19,22,26
X	FOURNIER S ET AL: "ROLE FOR LOW-AFFINITY RECEPTOR FOR IGE (CD23) IN NORMAL AND LEUKEMIC B-CELL PROLIFERATION" BLOOD, vol. 84, no. 6, 15 September 1994, pages 1881-1886, XP000609085 see page 1883, right-hand column, last paragraph - page 1884, right-hand column, line 16 ---	1-3,5, 11-15, 17-19
X	FISCHER, G. ET AL.: "Lymphoma models for B cell activation and tolerance. X. anti-mu-mediated growth arrest and apoptosis of murine B cell lymphomas is prevented by the stabilization of myc" J. EXP. MED., vol. 179, January 1994, pages 221-228, XP002089030 see page 225, right-hand column, line 10 - page 226, left-hand column, line 36 see page 227, left-hand column, last paragraph --- -/--	1-3,5, 11-15, 17-19, 22,26

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. Ional Application No
PCT/US 98/17261

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ROSS J: "Control of messenger RNA stability in higher eukaryotes" TRENDS IN GENETICS, vol. 12, no. 5, May 1996, page 171-175 XP004037270 see the whole document ---	1-46
A	NELLEN, W. ET AL.: "Mechanisms of antisense mediated mRNA stability control." JOURNAL OF CELLULAR BIOCHEMISTRY SUPPLEMENT, (1993) VOL. 0, NO. 17 PART E, PP. 197., XP002089031 see abstract S111 see abstract	46
O,A	& KEYSTONE SYMPOSIUM ON GENETICALLY TARGETED RESEARCH AND THERAPEUTICS: ANTISENSE AND GENE THERAPY KEYSTONE, COLORADO, USA APRIL 12-18, 1993, ---	
A	BELASCO J G ET AL: "Mechanisms of mRNA decay in bacteria: a perspective." GENE, (1988 DEC 10) 72 (1-2) 15-23, XP002089032 see abstract see page 20, line 1 - line 18 ---	
P,X	LIPMAN D J: "Making (anti)sense of non-coding sequence conservation." NUCLEIC ACIDS RESEARCH, (1997 SEP 15) 25 (18) 3580-3., XP002089033 see the whole document -----	1-46

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/17261

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-45 (as far as in vivo methods are concerned) is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 98/17261

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9711085 A	27-03-1997	AU 7073096 A	09-04-1997